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Do favored cuticular hydrocarbon profiles signal fertility? Analysis of *Gryllus firmus* and *Gryllus pennsylvanicus*

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Abstract

In *Gryllus*, mate choice is known to exist in both sexes. Because of the added cost of mate selection, most models assume that mate choice has benefits that outweigh these costs. *Gryllus firmus* and *G. pennsylvanicus* have sexually dimorphic cuticular hydrocarbon (CHC) profiles: Males have a relatively homogenous and simple profile, whereas females are more variable, and some individuals resemble the profile of males ("male-like" females). Previous studies have shown that males in captivity exhibit mate choice and prefer females with a more male-like profile, courting and mating with them more promptly. In this study, both species were used to test whether CHC serve as a signal of female fertility and whether females with a male-like CHC profile mate more often in the field. We captured field-mated females, scored their CHC profiles, and used seven highly polymorphic microsatellites to assign paternity to their broods. We report on the number of sires for field-caught females and demonstrate that male-like females are not more fertile and do not mate more often in the field. We also show that the allocation of sperm does not seem to follow a fair raffle: A few males dominate the brood composition of field-caught mated females.

Keywords

Fair raffle, field cricket, mating pattern, microsatellites, multiple mating, sexual selection

Introduction

Mate choice is costly, and many models assume that benefits must justify the costs (Andersson and Simmons 2006). Among models assuming benefits, "direct effects" and "good genes" (Hamilton and Zuk 1982) models of sexual selection are the most common. Direct benefits assume there is a phenotypic benefit, such as territory or even fertility, while the "good genes" model proposes that individuals choose characteristics that are "honest signals" of fitness. These two models, however, can be intertwined, since direct benefits (like ability to maintain a large territory or high fertility) might ultimately be explained by good genes (Kirkpatrick and Ryan 1991).

Traditionally, female mate choice has been regarded as the primary driver of sexual selection because reproduction costs are much higher for females. However, recent studies have also pointed to the critical role that male mate choice plays in mating success (Edward and Chapman 2011, Yukilevich 2012, Shahandeh and Turner 2020). Even in males, reproduction is costly and often results in a shortened lifespan due to the energy input into sexual signaling, sperm competition, and increased risk of exposure to predation or parasitoids (Cade 1975, Noguera 2019). Male mate choice can be observed through differential reproductive effort (e.g., courtship) based on female aspects such as size, species, and relatedness, which can be honest indicators of fitness (Simmons 1989, Gage and Barnard 1996, Howard and Blomquist 2005, Bateman and Fleming 2006, Maroja et al. 2014).

In this study, we investigated fertility in field-caught females of the hybridizing field crickets *Gryllus firmus* Scudder, 1902 and *G. pennsylvanicus* Burmeister, 1838. These crickets form a well-characterized mosaic hybrid zone and are large, locally abundant, and easy to manipulate, making them good models for studying sexual selection (Larson et al. 2013). Like many other insects, these crickets use cuticular hydrocarbons as chemical signals for mating (Maroja et al. 2014, Heggeseth et al. 2020). In *G. firmus* and *G. pennsylvanicus*, cuticular hydrocarbons are sexually dimorphic: Male CHC profiles are virtually identical both within and between species, whereas females are much more variable within and between species, with some females exhibiting a profile similar to that of a male ("male-like" female) (Maroja et al. 2014, Heggeseth et al. 2020). While these CHC profiles are likely influenced by both genes and the environment, there seem to be differences between species (Maroja et al. 2014). We also note that crickets hatched and raised in the lab exhibit profiles similar to those of their respective species (unpublished data).

Previously, we demonstrated that in captivity, *G. firmus* and *G. pennsylvanicus* males courted females with a male-like cuticular hydrocarbon profile more readily than females with a typical cuticular hydrocarbon profile, pointing to the existence of male mate choice (Maroja et al. 2014, Heggeseth et al. 2020) within these

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species. The reason for this male mate choice, if any, was not apparent, as these different types of females were indistinguishable with respect to size or longevity. However, our prior work did not quantified differences in fertility or any other metrics of fitness.

We expected that if males in the field also courted male-like females more intensely, these females would mate more often and have more males siring their progeny. Furthermore, if male mate choice can be explained by benefits (direct or indirect), fertility differences between females with different CHC profiles would be a good phenotype to focus on. We thus investigated whether the females exhibiting a male-like CHC profile mate with more males in the field and whether they are more fertile. Our results reveal no advantages for male-like females in terms of fertility or mating frequency in the field (paternity of offspring), suggesting that cuticular hydrocarbon profiles might not serve as "honest signals" for good genes. We also demonstrate that the paternity counts for each female deviate significantly from the fair lottery, where fertilizations are expected to be similar for each male, a pattern previously reported for field crickets (Simmons 2001, Simmons et al. 2003, Larson et al. 2012). Here, we discuss the potential reasons behind these results.

Methods

Field collection.—We collected *G. firmus* and *G. pennsylvanicus* crickets from allopatric populations in Guilford, CT (41.268501, -72.668212, *G. firmus*) and Pownal, VT (42.755941, -73.233340, *G. pennsylvanicus*) on September 5–8, 2020. The collection took place after most females had been adults for at least 2 weeks and up to a month (any late-instar or recently molted females were released); in our past experience in the field, almost all individuals are adults by mid-August; we thus assumed that by early September females would have mated multiple times. These adult field-mated females were individually placed in plastic shoebox cages (30 × 16 × 9 cm) with a tall 10 cm petri dish filled with moist coconut fiber for oviposition. All the crickets were kept in a 25 °C environment with ad libitum food (Fluker's orange Cube Complete Cricket Diet and Fluker's High-Calcium Cricket Diet, Fluker's Cricket Farm, Inc., Port Allen, LA, USA) and water. Males of both species were also captured for cuticular hydrocarbon analyses.

Fertility trials.—Field-captured mated females were kept alone with *ad libitum* water and food and oviposition dishes for two months. A total of 47 *G. firmus* females and 39 *G. pennsylvanicus* females were used to test fertility differences between females with different CHC profiles. The females remained in the box with an oviposition Petri dish and *ad libitum* access to food and water for another two months. At the end of the oviposition period, we euthanized the females for cuticular hydrocarbon (CHC) and DNA extraction. All of the eggs laid by each female over her lifetime were kept in the same petri-dish—we could not separate offspring from early and late life. In the field, females die after the first frosts; thus, we euthanized the females in early November. Then, to simulate diapause, we incubated Petri dishes containing the eggs at 6 °C until early January. The moisture content of the oviposition media was checked once a week. On January 12, 2021, the oviposition dishes were placed for incubatiom at 25 °C until hatching. The nymphs were allowed to grow for approximately 10 days, at which time they were preserved in ethanol and counted.

Paternity testing.—A total of 29 *G. firmus* and 19 *G. pennsylvanicus* females were used for paternity analyses. The remaining 18 *G. firmus* and 20 *G. pennsylvanicus* were excluded because of a lack of DNA, failure in multiple PCRs (7 *G. firmus* and 14 *G. pennsylvanicus*), or production of fewer than 10 offspring (11 *G. firmus* and 6 *G. pennsylvanicus*). Notice that failure to produce offspring after diapause in lab settings is relatively common even for females observed mating (e.g., Maroja et al. 2009).

Genomic DNA was extracted from the hind femurs of all mated females using a DNeasy® Blood and Tissue Extraction Kit. For the early-instar nymph offspring, DNA was extracted from the whole body via a modified HotSHOT procedure, excluding 0.2 mM disodium EDTA for the alkaline lysis reagent (Truett et al. 2000). The female and a maximum of 48 offspring were genotyped for each brood with seven locus-specific microsatellite primers developed from *G. pennsylvanicus* (Larson et al. 2012). The loci Gr14, Gr54, Gr112, Gr115a, Gr116, Gr143, and PGI (see Table 1) were used, and all forward primers were labeled with fluorescent dye. To perform the PCR, we followed the QIAGEN Type-It Microsatellite Kit protocol. The PCR products were then diluted 1:1 with Barnstead water. We added 0.3 µL of Genescan LIZ-500 size standard and 18 µL of formamide to 2 µL of diluted PCR product. The PCR samples were sent for fragment analysis to the Cornell Institution of Biotechnology (Applied Biosystem 373oxl instrument). We used Geneious Prime (Geneious 2024.0.7) to visualize peaks and call alleles.

After calling microsatellite peaks, the data were transferred into CERVUS (Kalinowski et al. 2007) to calculate allele frequencies. Subsequently, the data and allele frequencies were transferred into COLONY (Jones and Wang 2010) to calculate the inferred parentage from our multilocus genotype data. We ran each brood through the program individually, using seven loci, known allele frequencies, known female progenitor genotypes, female polygamy, and maternal sibships among all nymphs. We set the model to a dioecious outbreeding system. Each run was set to run once on a medium length using a single thread. The optimization algorithm was run assuming 1%, 10%, and 25% error rates for the genotype calls.

Cuticular hydrocarbon (CHC) analysis.—We extracted CHCs from all adults from both the fertility and paternity experiments. Females had been raised on a standard diet and at a standard temperature (lab) for months prior to CHC extraction. We placed whole crickets (euthanized by freezing at -20 °C for 10 minutes) into a 3-dram glass vial and filled it with 5 mL of HPLC-grade hexane for 5 minutes. Once the CHCs were suspended in hexane, all the samples were evaporated

Table 1. Information for the microsatellite loci used in the paternity analyses. Fluorescent tag was added to forward primers. Reverse primers had the 5'tag "GTTTCTT" (Brownstein et al. 1996).

Locus	GenBank	Primer sequence (5'–3')	Fluorescent tag
PGI	JN379460	F: GAATGCATACATCAGTGTCATGAACA R: TGACTCAAAATAAGCATTATTTCAGC	6-FAM
Gr143	JN375328	F: CTGCCGCATTCACCAATCATTCAACTAT R:CAACCAAGGGGCAAAATGAGTCAAACTT	VIC
Gr14	JN375323	F: AGAAACGCTAGGTTAGTACTATTGGTAA R: TCGCGAAATTCGAATTGTATC	NED
Gr116	JN375327	F: CACACGTGATATACCGACAACACT R: GCTTTAACCCCCTAGCAGAATAAT	PET
Gr115a	JN375326	F: TCGCCAAGACTTACGCTCAG R: CCTCCTCGGCACAGACG	6-FAM
Gr54	JN375324	F: CGCGCCATCTTTTTCTTATTCATTCTTCT R: CGTTCCTCTCCGCTGCCGTCGTA	VIC
Gr112	JN375325	F: GAATAATAAAAGCGCAAGGATA R: CACCATTACACGTACTCACTCG	PET

and resuspended in 350 µL of hexane with a 75-ppm concentration of docosane as a reference control. The docosane acted as a positive control and a reference point for standardizing other CHC compounds in the sample (every individual had this same internal control). We then transferred the CHC samples into 2 mL clear glass vials and analyzed them via an Agilent Technologies (AT) 7820B GC system with an Agilent 19091S-433UI 30 m × 250 µm × 0.25 µm column. For the GC–MS method, 2 µL of each sample was injected into a split mode set at a split ratio of 10:1. After a 1.7-minute solvent delay, the initial temperature of the column was raised from 200°C to 250°C over a 2-minute period, then to 260°C over a 10-minute period, followed by a 1-minute ramp to 300°C, where the temperature was held for 13 minutes. The MS instrument was operated in electron impact mode (70 eV) with a mass range of 45–550 m/z.

Using Masshunter Qualitative Analysis Version B.10, we filtered the top 20 peak areas within each individual sample. These data were then transferred into R for peak alignment on the basis of retention time. The first step is to linearly shift retention times on the basis of the reference point within each sample. The second step involves using agglomerative hierarchical clustering with complete linkage to merge homologous peaks by comparing retention times across samples and grouping similar retention times while controlling the maximum peak width. Since male CHC profiles are less variable across samples (Maroja et al. 2014, Heggeseth et al. 2020), we first aligned the peaks in the male samples to determine the appropriate maximum peak width parameter to be 0.3 in retention time. This parameter was then applied to all individual samples, and the remaining retention times were merged with a maximum peak width of 0.3. This process resulted in 26 final peaks across the entire analyzed population.

Statistical analyses

CHC profiles.—We used R (version 4.4.1) for all the statistical analyses (R Core Team 2024). To assign CHC profiles as "male-like" or "non-male-like," we used the aligned CHC data for all samples, including females of both species from both the fertility and fecundity experiments as well as males (a total of 63 males and 157 females). We used principal component analysis on the relative areas of the 26 peaks to visualize the CHC profiles. The first five principal components, which maintain 64% of the original variability, were used to cluster female samples into four CHC profile groups via the partitioning around the medoids algorithm in the cluster R package (Maechler et al. 2024).

Fertility analysis.—Having classified females as male-like or non-male-like within *G. firmus* or *G. pennsylvanicus*, we tested for differences in fertility. As some females produced no offspring, we used logistic regression to model whether a male-like CHC profile and species are associated with having any offspring. Second, we modeled the number of offspring via a Poisson regression model, allowing the CHC profile and species to explain the variation in the number of offspring. Wald tests were used to test differences between CHC profiles within each species. To test for differences between species (not considering the cuticular hydrocarbon profile), we refit the models with only species and used a Wald test.

Paternity analysis.—We used the inferred parentage package from COLONY (Jones and Wang 2010) to calculate the estimated number of sires contributing to broods and the distribution of offspring per potential sire. We used Poisson regression to model the estimated number of sires as a function of the male-like CHC profile and species and fitted models separately for each dataset produced by COLONY for each error rate. Additionally, we fit Poisson models with only species and used Wald tests to compare estimated paternity.

To study the utilization of sperm within each brood, we considered how many offspring each potential sire contributed to. If sperm utilization followed a fair raffle, we would expect the distribution of offspring per sire to be generated from a discrete uniform distribution. For each brood, we tested the fair raffle hypothesis with a chi-square test. We summarize the individual tests and ran an overall sum test that assumes that the sum of independent chi-square variables follows a chi-squared distribution with degrees of freedom equal to the sum of the individual degrees of freedom. We combined information across all broods (Koziol and Perlman 1978).

Results

CHC profiles.—The partitioning around the medoids algorithm identified 4 clusters to explain the full variation in CHC profiles–but since our focus is on ML-like profiles, we utilized just that cluster for the analyses. Of the 157 females, 21 *G. firmus* and 19 *G. pennsylvanicus* were in the male-like cluster (see Fig. 1). Not all females scored for CHC analyses were part of the fertility analyses.

Fertility analysis.—We scored 44 *G. firmus* broods and 38 *G. pennsylvanicus* broods. The average number of offspring among male-like *G. firmus* (n = 11) was 74.2 (se = 37.3), which was slightly lower than the 95.3 (se = 18.5) for those without male-like CHC profiles (n = 33). A similar pattern was observed among male-like *G. pennsylvanicus* (n = 11), with an average of 132 (se = 50.4) offspring, compared with 183 (se = 21.8) offspring among those that did not have a male-like CHC profile (n = 27, see Fig. 2). While we do not have strong evidence to suggest that CHC profiles impact whether a female has any offspring (Wald test, df = 1, p = 0.09), we found significant differences in the number of offspring between females with a male-like or typical cuticular hydrocarbon profile for both *G. firmus* (Wald test, df = 1, p < 0.001) and *G. pennsylvanicus* (Wald test, df = 1, p < 0.001); in both species, male-like females produced fewer offspring.

Among the species, *G. firmus* produced fewer offspring than did *G. pennsylvanicus* (90 (se = 16.6) for *G. firmus* and 168 (se = 21.2) for *G. pennsylvanicus*, Wald test, df = 1, p < 0.001), which contrasts with observations in prior studies in which *G. firmus* was usually more fertile (Maroja et al. 2009).

Paternity analysis

Differences between females with male-like CHC profiles.—Here, we report the estimated number of sires produced by COLONY (Jones and Wang 2010), assuming a 10% error rate (error rates of 1% and 25% were qualitatively equivalent to the 10% error rate; see methods). Because some sires contributed to few offspring, or sometimes a single one, our estimates represent the minimum number of sires; the female might have mated with more males but failed to produce offspring from these males.

We scored 29 field-caught *G. firmus* broods and 19 field-caught *G. pennsylvanicus* broods and estimated paternity for a maximum of 48 offspring. No significant difference was found between the estimated number of sires contributing to broods from females with a male-like (8 *G. firmus* and 4 *G. pennsylvanicus*) or non-male-like cuticular hydrocarbon profile for either *G. firmus* (male-like mean: 6 (se = 0.5) vs non-male-like mean: 5.5 (se = 0.4), Wald test, df = 1, p = 0.6) or *G. pennsylvanicus* (male-like mean: 8.8 (se = 0.8) vs non-male-like mean: 8 (se = 0.8), Wald test, df = 1 p = 0.6; see Fig. 3).

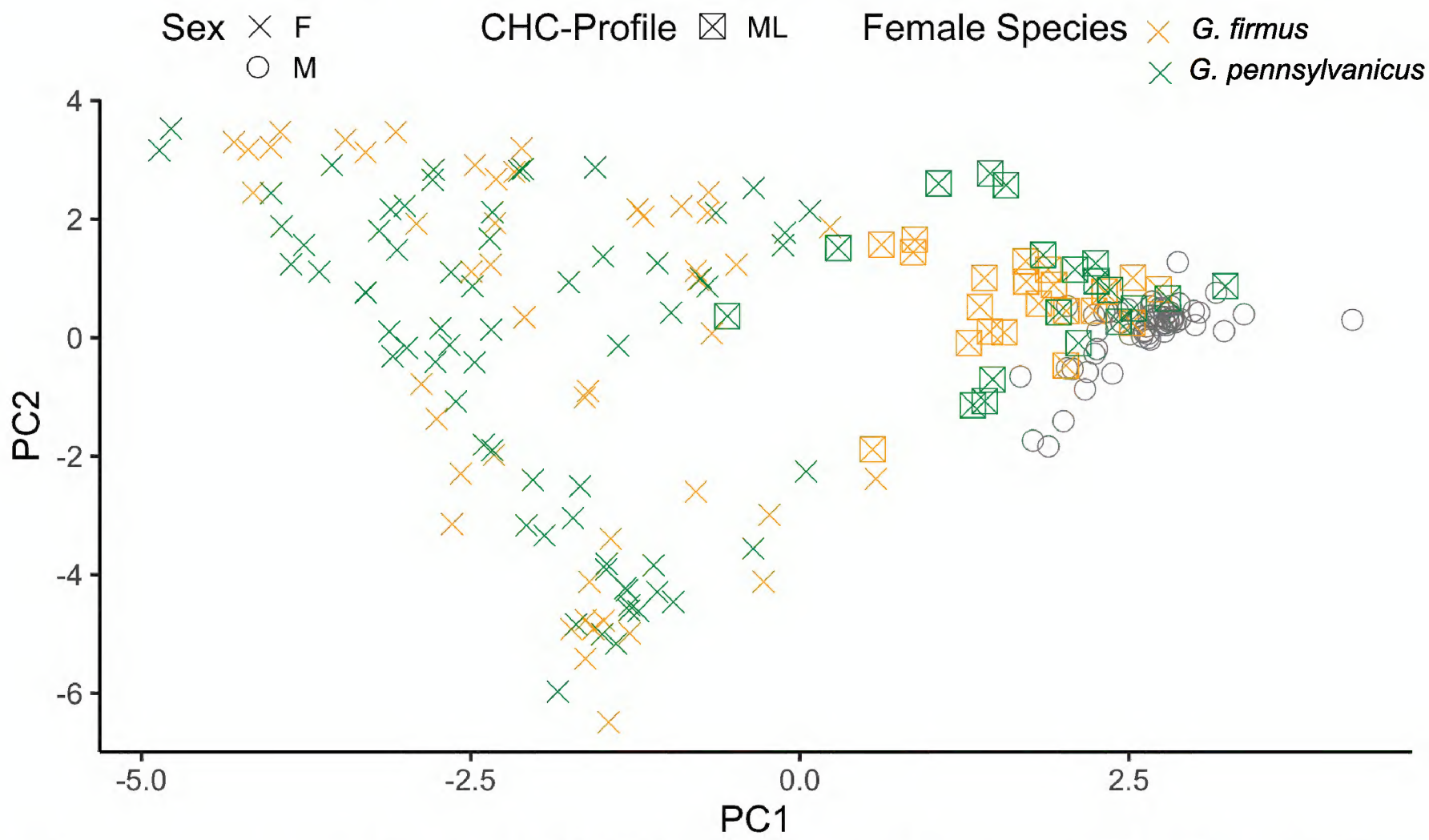

Fig. 1. Principal component analyses of CHC profiles for *G. firmus* and *G. pennsylvanicus* males and females. Females (X symbol) with the male-like category identified with the partitioning around the medoids algorithm (considering the five first principal components) are shown with a square. Orange/green represents *G. firmus* and *G. pennsylvanicus*, respectively, and males (O symbol) of both species are shown in gray.

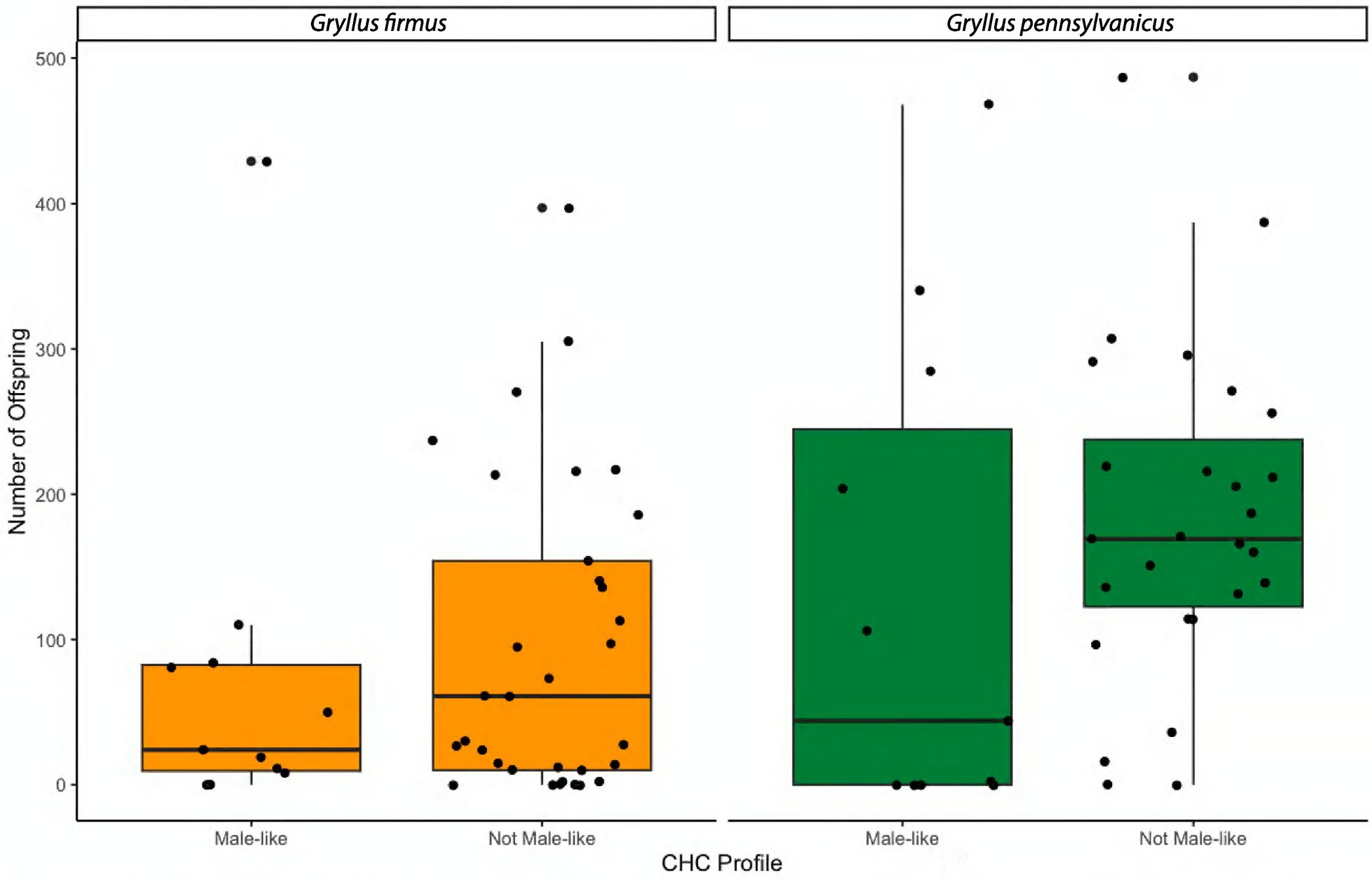

Fig. 2. Number of offspring for male-like and non-male-like females of both species. In both *G. firmus* and *G. pennsylvanicus*, male-like females produced significantly fewer offspring.

Among the species, *G. firmus* broods had fewer contributing males than *G. pennsylvanicus* did (means of 5.6 (se = 0.3) for *G. firmus* and 8.2 (se = 0.7) for *G. pennsylvanicus*, Wald test, df = 1, p = 0.001).

Fair-raffle utilization of sperm.—We found that the likelihood of siring offspring was not evenly distributed across males. The overall sum test (df = 271, p ~ 0) provided very strong evidence that the fair-raffle hypothesis does not hold within each species. Among the individual chi-square tests, approximately 81% of broods have offspring distributions that are different enough from a fair raffle to reject at the 0.05 significance level (chi-square test, Fig. 4). That is, there is a substantial deviation of the fair raffle if we assume that each male contributed the same amount of sperm (see discussion).

Discussion

Although previous results indicate that both *G. firmus* and *G. pennsylvanicus* males court females with male-like cuticular hydrocarbon profiles more readily in the laboratory (Heggeseth et al. 2020), we found no evidence that this male mate choice had an effect on mating rates in the field or that the favored females had an advantage in fertility. We found no evidence that *G. firmus* or *G. pennsylvanicus* females with male-like CHCs have greater fertility than typical females of each species; in fact, male-like females had slightly lower fertility. We also did not find evidence that females with male-like cuticular hydrocarbons mated more often in the field; the average number of mates was similar for both female types. Surprisingly, we found that while previous lab-based studies have suggested that sperm utilization in crickets follows a fair raffle (Simmons 2001, Simmons et al. 2003, Larson et al. 2012), we did not observe an even offspring spread among sires. Instead, one or a few males often dominate the paternity of a given female's brood.

No evidence that cuticular hydrocarbons are related to fertility.—Although there are relatively few studies of cuticular hydrocarbons in field crickets, it is known that many species do use CHCs for mate choice: The majority of the studies report female mate choice (Thomas and Simmons 2009, Steiger et al. 2013, Steiger et al. 2015). To the best of our knowledge, *G. firmus* and *G. pennsylvanicus* are the first

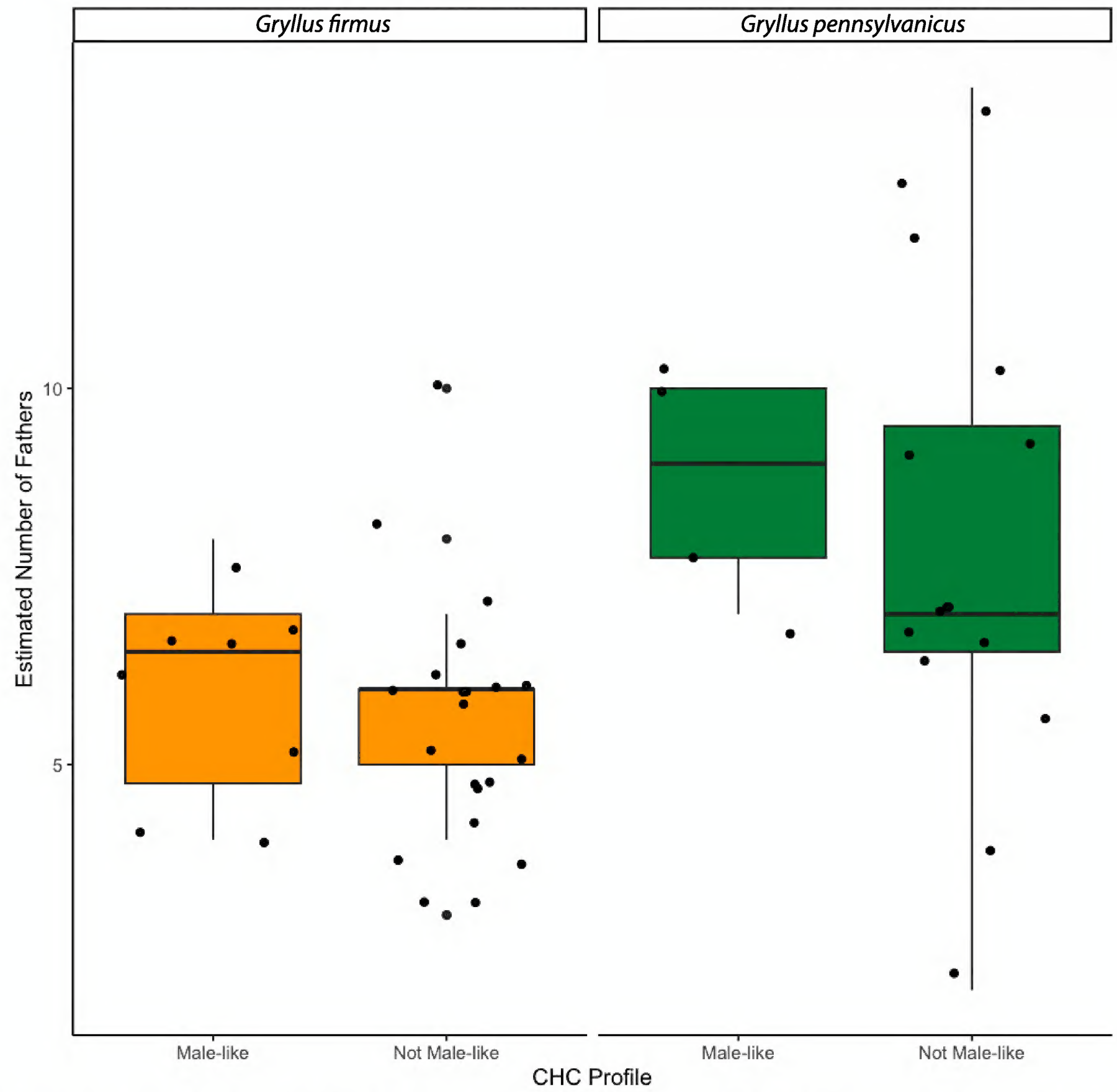

Fig. 3. Estimated number of sires for male-like and non-male-like females from each species. There were no significant differences between the CHC categories, but there was a significant difference between the two species.

species reported to have male mate choice based on cuticular hydrocarbons (Maroja et al. 2014). Furthermore, unlike *Laupala* (Mullen et al. 2008), *G. firmus* and *G. pennsylvanicus* have largely overlapping CHC profiles between species: Males of the two species have virtually identical CHC profiles, whereas females have extremely variable and partially overlapping profiles with some females exhibiting profiles identical to that of males (male-like CHC profiles, Fig. 1).

Previous laboratory-based studies have suggested that female crickets with a male-like cuticular hydrocarbon profile were courted more readily than their counterparts in both species (Heggeseth et al. 2020). Because mate choice is expensive, most models assume that there are hidden benefits behind the choice, raising the possibility that male-like CHC profiles might be indicators of higher fitness. Fitness advantages could take many forms, from better territories and food access to better survivorship or higher fertility. Given that female crickets do not guard a territory but do have large variance in fertility in laboratory conditions with *ad libitum* food (Maroja et al. 2009), we tested whether fertility was associated with CHC profiles. We found that the favored male-like females had lower fertility than the other females (Fig. 2). Thus, the data failed to confirm that male choice is driven by higher fertility. Of course, these females could still have higher fitness for traits we did not measure. We did not measure survival or fertility in the field or offspring fitness and could not measure differences in access to food in the field. However, our results suggest that male crickets in laboratory trials might prefer male-like females for reasons unrelated to fertility at least in conditions when food is abundant (i.e., laboratory setting). It is possible that the observed male mate choice might be explained by sensory bias.

If the ancestral CHC profile of field crickets was sexually monomorphic and the diversification of females away from the typical male CHC profile is the more recent derived condition, then males might still have a sensory bias toward the ancestral, male-like profile. In dragonflies, andromorphic females can escape male harassment better than their gynomorphic counterparts (Fincke 2015). This is the opposite of what we observed in *G. firmus* and *G. pennsylvanicus*, where males court females with andromorphic cuticular hydrocarbon profiles more promptly than those with other cuticular hydrocarbon profiles (Heggeseth et al. 2020). Perhaps, in these species, a diversification of female CHC profiles could have led to a reduction in male harassment by deviating from what males were selected to detect. Interestingly,

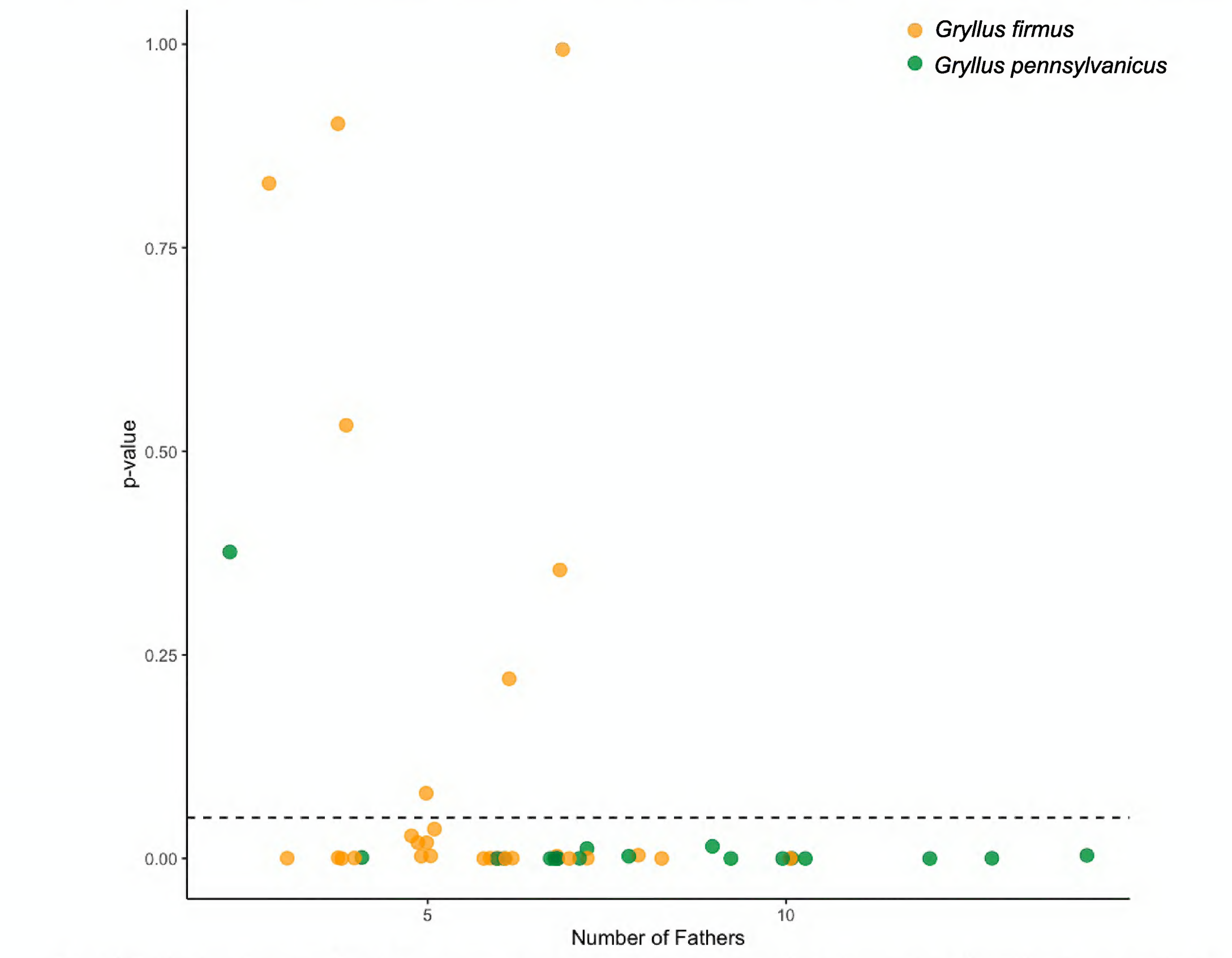

Fig. 4. Distribution of p-values for *G. firmus* (orange) and *G. pennsylvanicus* (green) broods assuming a fair raffle of sperm utilization. The 48 points representing broods are jittered to minimize overlap.

our unpublished data suggest that several other *Gryllus* species and outgroups are sexually monomorphic, with sexual dimorphism seemingly evolving at the base of *G. firmus* and *G. pennsylvanicus* divergence (LSM unpublished data).

No differences in paternity between CHC types.—We hypothesized that *G. firmus* and *G. pennsylvanicus* female crickets with a male-like cuticular hydrocarbon profile would mate more often because, in the laboratory, males courted females with male-like cuticular hydrocarbon profiles more readily (Heggeseth et al. 2020). Thus, we expected that broods from field-caught mated females with male-like CHC profiles would have more contributing sires. Our data did not confirm this expectation. There was no significant difference between females with or without male-like cuticular hydrocarbon profiles in either *G. firmus* or *G. pennsylvanicus* (Fig. 3); if a difference exists, it is very small, and we did not have the power to detect it. One caveat of our analyses is that we estimate a *minimum* number of sires because some of the males contributed to as few as one offspring; therefore, if we failed to detect many males, our power would be decreased.

There was a difference in the number of males that sired offspring between species. While *G. pennsylvanicus* broods were on average sired by 8.2 males (se = 0.7) males, *G. firmus* broods were sired by 5.6 (se = 0.3) males (Wald test, df = 1, p = 0.001). This result might be surprising considering the faster courtship times of *G. firmus* crickets in laboratory mating trials (Maroja et al. 2009, Heggeseth et al. 2020). These differences could also be a consequence of development time differences: During collection in early September, we observed that while all the *G. pennsylvanicus* crickets were fully mature, there were occasional last-instar *G. firmus* nymphs in the Guilford CT field population (while we observed 3 out of more than 30 females, these females were not collected). If *G. firmus* females were, on average, younger adults at collection time, they would have had less time to mate in the field; developmental time differences were also reported in a previous study (Harrison 1985).

Utilization of sperm: not a fair raffle.—Based on prior studies (Simmons 2001, Simmons et al. 2003, Larson et al. 2012), we expected that the crickets would follow a fair raffle principle, in which sperm from all males would mix and have an equal chance at fertilization (Parker et al. 1990). Previous studies have purported that sperm mix within the spermatheca of female crickets and that mating of two *G. pennsylvanicus* males with a female suggests the mixing of sperm and a fair raffle (Britch et al. 2007, Larson et al. 2012). Here, we did not observe a fair raffle (Fig. 4); instead, paternity was biased toward a few males in each brood, indicating a loaded raffle (Parker et al. 1990).

While the cause of the loaded raffle in field-mated females is impossible to fully explain due to a lack of controlled mating, there are several potential explanations that could make our results compatible with a fair raffle. There could be differences in male fitness, so each male does not supply the same quantity or quality of sperm. In *Teleogryllus oceanicus*, older males have a greater proportion of viable sperm and thus sire more offspring (García-González and Simmons 2005). Furthermore, the mass of the spermatophore and the quantity of sperm from a given male could also impact paternity. As adult males develop, the spermatophore size increases and is impacted by mating frequency (more matings lead to smaller spermatophores) (Duplouy et al. 2018). In some species, males adjust spermatophore size based on perceived sperm competition based on CHC, decreasing the number of sperm for multiple inseminated females (House et al. 2024). Thus, even if females mate once with each male, the quality and/or quantity of sperm could vary dramatically depending on age, mating frequency, and external factors such as food availability and competition. Finally, we do not know if females repeatedly mate with the same males, which would increase the number of sperm available for that male even if all males had equal fitness.

We also cannot eliminate the possibility of sperm clumping, leading to a "sloppy mix" within the spermatheca (Harvey and Parker 2000). If the spermatophores clump within the spermatheca, nonrandom mixing could occur, leading to a loaded raffle. Either clumping could lead to one male disproportionately fertilizing a majority of the eggs or, conversely, fertilizing very few eggs because it fails to mix with the other sperm.

Other potential mechanisms of sperm competition and utilization, such as indirect displacement, stratification, and cryptic female choice, could also explain these results. However, owing to the morphology (round) of the cricket spermatheca, these mechanisms are unlikely to lead to the observed paternity skew (Parker and Pizzari 2010).

Conclusion

We found that the preferred females, those with male-like cuticular hydrocarbon profiles, did not have higher fertility than other females in either *G. firmus* or *G. pennsylvanicus*. Furthermore, the field-caught male-like females did not produce offspring sired by more males despite the faster courtship times observed in laboratory mating experiments. We also observed a loaded raffle in the paternity distribution for both *G. firmus* and *G. pennsylvanicus*, contrary to our predictions and prior work indicating a fair raffle in these species. Further work should be done to identify the mechanisms that explain the discrepancy between the field-caught and captive crickets and to elucidate the causes of the loaded raffle.

Availability of data and materials

The datasets and analyses (R scripts) supporting the conclusions of this article are available in the DRYAD repository: https://doi.org/10.5061/dryad.v9s4mw768.

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